PATENT
3587-0110P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	MAY, Choo Yuen et al.	Conf.:	Unassigned
Appl. No.:	10/642,596	Group:	Unassigned
Filed:	August 19, 2003	Examiner:	UNASSIGNED

For: EXTRACTION OF PALM VITAMIN E, PHYTOSTEROLS AND SQUALENE FROM PALM OIL

L E T T E R

October 22, 2003

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Under the provisions of 35 U.S.C. § 119 and 37 C.F.R. § 1.55(a), the applicants hereby claim the right of priority based on the following application:

Country	Application No.	Filed
MALAYSIA	PI 2002 3069	August 20, 2002

A certified copy of the above-noted application is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fee required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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KM/mzk
3587-0110P

Attachment

(Rev. 09/30/03)

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Int ll ctual Prop rty Corporation of Malaysia

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3587-0110P
10/642,596
AUGUST 13, 2003
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PATENT APPLICATION NO: PI 2002 3069

This is to certify that annexed hereto is a true copy from the records of the Registry of Trade Marks and Patents, Malaysia of the application as originally filed which is identified therein.

By authority of the
REGISTRAR OF PATENTS

ABDUL RAHMAN RAMLI
(CERTIFYING OFFICER)
4 September 2003

KEMENTERIAN PERDAGANGAN DALAM NEGERI
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CERTIFICATE OF FILING

APPLICANT	:	MALAYSIAN PALM OIL BOARD (MPOB)
APPLICATION NO	:	PI 20023069
REQUEST RECEIVED ON	:	20/08/2002
FILING DATE	:	20/08/2002
AGENT'S/APPLICANT'S FILE REF.	:	PK/P866/MPOB/2002

Please find attached, a copy of the Request Form relating to the above application, with the filing date and application number marked thereon in accordance with Regulation 25(1).

Date : 29/08/2002

.....
(ROZILEE B. ASID)
for Registrar of Patents

To : P. KANDIAH
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FF 09

Patents Form N . 1 PATENTS ACT 1983 REQUEST FOR GRANT OF PATENT (Regulation 7(1)) To: The Registrar of Patents Patent Registration Office Kuala Lumpur Malaysia	**For Official Use** APPLICATION RECEIVED ON: ..... Fee received on: ..... Amount: ..... *Cheque / Postal Order / Money Order / Draft / Cash No.: ....
Please submit this Form in duplicate together with the prescribed fee.	Applicant's file reference: **PK/P866/MPOB/2002**

THE APPLICANT(S) REQUEST(S) THE GRANT OF A PATENT IN RESPECT OF THE FOLLOWING PARTICULARS:

TITLE OF INVENTION: **EXTRACTION OF PALM VITAMIN E, PHYTOSTEROLS AND SQUALENE FROM PALM OIL**

II. APPLICANT(S) (the data concerning each applicant must appear in this box or, if the space is sufficient, in the space below)

Name: **MALAYSIAN PALM OIL BOARD (MPOB)**

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- Permanent residence or principal place of business:

AS ABOVE

Telephone Number (if any)	Fax Number (if any)
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Additional Information (if any)

III. INVENTOR

Applicant is the inventor: Yes [ ] No [X]

If the applicant is not the inventor:

Name of the inventors:
1. **Dr. Choo Yuen May (IC No. 551227-07-5578)**
2. **Harrison Lau Lik Nang (IC No. 750810-13-5883)**
3. **Dr. Ma Ah Ngan (IC No. 480517-08-5357)**
4. **Dr. Yusof Barison (IC No. 480620-05-5359)**

Address of inventors: **All are of**
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43000 Kajang, Selangor, Malaysia

A statement justifying the applicant's right to the patent accompanies this Form:

Yes [X] No [ ]

Additional Information (if any)

IV. AGENT OR REPRESENTATIVE:

Applicant has appointed a patent agent in accompanying Form No. 17. (to follow)

Yes [ ] No [ ]

Agent's Registration No: **PA 90/019**

Applicants have appointed **P. Kandiah** to be their common representative

IV. DIVISIONAL APPLICATION

This application is a divisional application Yes [ ] No [ ]

The benefit of the

Filing date [ ] Priority date [ ]

Of the initial application is claimed in as much as the subject matter of the present application is contained in the initial application identified below: -

Initial Application No:

Date of filing of initial application:

VI. DISCLOSURES TO BE DISREGARDED FOR PRIOR ART PURPOSES

Additional information is contained in supplemental box:

(a) Disclosure was due to acts of applicant or his predecessor in title

Date of disclosure: ☐

(b) Disclosure was due to abuse of rights of applicant or his predecessor in title

Date of disclosure: ☐

A statement specifying in more detail the facts concerning the disclosure accompanies this Form

Yes ☐ No ☐

Additional Information (if any)

VII. PRIORITY CLAIM (if any)

The priority of an earlier application is claimed as follows :

Country (if the earlier application is a regional or international application, indicate the office with which it is filed):

Filing Date:

Application No:

Symbol of the International Patent Classification:

If not yet allocated, please tick ☐

The priority of more than one earlier application is claimed:

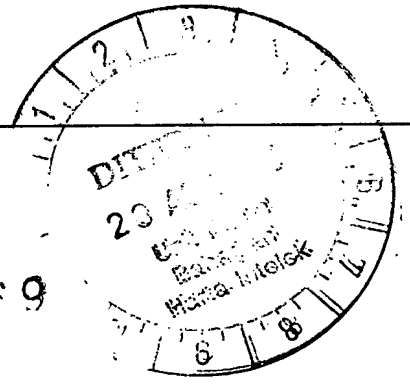
Yes ☐ No ☐

The certified copy of the earlier application(s) accompanies this Form:

Yes ☐ No ☐

If No, it will be furnished by (if requested by Registrar)

Additional Information (if any) :



VIII. CHECK LIST

A. This application contains the following:

1.	Request	**1**	Sheets
2.	Description	**16**	Sheets
3.	Claim	**3**	Sheets
4.	Abstract	**1**	Sheets
5.	Drawing	**2**	Sheets
	Total	**23**	Sheets

B. This Form, as filed, is accompanied by the items checked below:

(a)	signed Form No. 17	
(b)	declaration that inventor does not wish to be named in the patent	
(c)	statement justifying applicant's right to the patent	
(d)	statement that certain disclosures be disregarded (to follow)	
(e)	priority document (certified copy of earlier application)	
(f)	cash, cheque, money order, banker's draft or postal order for the payment of application fee	X
(g)	other documents (specify) -	

IX. SIGNATURE:..... .....

P. Kandiah
**(~~Applicant~~/Agent) (Date)

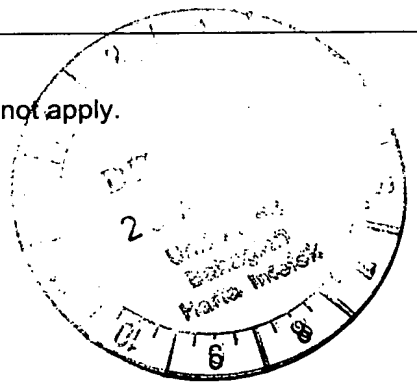
If Agent, indicates Agent's Registration No: **PA 90/019**

For Official Use

1. Date application received:

2. Date of receipt of correction, later filed papers or drawings completing the application:

* Delete whichever does not apply.
** Type name under signature and delete whichever does not apply.



20023069

EXTRACTION OF PALM VITAMIN E, PHYTOSTEROLS AND SQUALENE FROM PALM OIL

FIELD OF INVENTION

The invention relates to the extraction of phytosterols, squalene and vitamin E form crude palm oil. More particularly the present invention relates to an integrated process to extract phytosterols, squalene and vitamin E from crude palm oil.

BACKGROUND OF THE INVENTION

Palm oil contains 700-1000 ppm of vitamin E, 300-620ppm of phytosterols and 250-730ppm of squalene. The present invention relates to a process for the recovery of the natural occurring Vitamin E, phytosterols and squalene from crude palm oil.

Vitamin E is a group of natural occurring lipid soluble antioxidants, namely tocopherols and tocotrienols that are found in certain vegetable oils. The main occurrence of tocotrienols is in palm oil, wheat germ oil, coconut oil and corn oil. Tocotrienols possess higher antioxidant activity than tocopherols, which have been shown in biochemical studies (Serbinova et al., 1991, Pokorny, J 1987 and Jacobsberg et al 1978). As a predominant type of vitamin E constituting 80% of total vitamin E found in palm oil, tocotrienols have also been known to possess hypocholesteolemic effect (Tan et al 1991 and Qureshi et al 1991).

Phytosterols are structurally similar to cholesterol except they are alkylated at the 24 position in the side chain. The most abundant type of phytosterols by far found in plants are ß-sitosterol, stigmasterol and campesterol. These compounds are natural components of diet and are consumed in amounts of 100-500 mg/day with respect to US consumption (Weirauch, JL Gradner, JM 1978. Sterol content of foods of plant origin. J Am, Diet. Assoc. 73:39-47). Studies conducted employing ß-sitosterol were found to significantly reduce the amount of cholesterol in the blood (Farguhar, J W et al 1956. Circulation, 14,77-82). Palm oil is rich in phytosterols with 60% of ß-sitosterol and the remaining 38% is stigmasterol and campesterol. Therefore it provides a natural source of phytosterols for recovery.

Squalene is a major component in various deep-sea shark liver oils. It is a powerful antioxidant that can scavenge free radicals from the body before they start their debilitating effect. Trials have shown that where squalene is taken as a dietary supplement, evidence has shown that it has preventative effects against carcinogenesis.

Squalene presents as one of the minor components in palm oil. It could be recovered as a valuable antioxidant if presented in high concentration.

The related patents which have been filed include WO0009535, GB 531226, GB 549931, GB 531224 and EP 0541999. These patents concentrate in the recovery of vitamin E or vitamin E and phytosterols but not as an integrated process for the recovery of vitamin E, phytosterols and squalene together as described in this invention. The patented inventions only proceed with one stage vacuum distillation in which it does not serve for the removal of high molecular weight components as described in this invention. Therefore, it is an objective of this invention to provide a method for purifying and recovering of these valuable minor compounds namely vitamin E, phytosterols and squalene to their respective fractions with crystallized phytosterols at high purity.

SUMMARY OF THE INVENTION

The invention relates to an integrated process for the recovery of valuable palm oil phytonutrients more particularly vitamin E, phytosterols and squalene which comprises the steps of acid/alkaline catalysed esterification/transesterification process of palm oil with lower alkyl alcohol, multi-stage vacuum distillation of alkyl esters, saponification of the phytonutrients concentrate, crystallization of phytosterols and finally partitioning of vitamin E and squalene with organic solvents.

Crude palm oil was esterified in alkyl alcohol preferably methanol and ethanol using sodium hydroxide or potassium hydroxide as catalyst to substitute the glycerol portion of glycerides with alkyl groups for the production of alkyl esters and glycerol. The type of alkyl alcohols used depending on the volatility of the alkyl esters produced in which the lower boiling point alkyl esters with shorter alkyl chain length are preferable in this case.

The lower boiling alkyl esters were subjected to multi-stage vacuum distillation, preferably three stage short path distillation (SPD) at different operating conditions as described below. The first short path distillation served the purpose to distil about 90% of the bulk esters with minimal amount of vitamin E, phytosterols and squalene being distilled over to the distillate. The applied short path distillation conditions are temperature ranging from 70°C to 120°C and pressure ranging from 10mTorr to 50mTorr. The phytonutrients enriched residue was then subjected to second short path distillation in the removal of all the impurities and colouring materials/pigments including carotenes, phospholipids, glycolipids, waxes, oxidized

products and other long chain hydrocarbons. The operating conditions are temperature ranging from 130°C to 200°C and pressure less than 1mTorr. The distillate from the second short path distillation was subsequently subjected to the third short path distillation to produce vitamin E, phytosterols, squalene and monoglycerides concentrates in a mixture with operating temperature less than 130°C and pressure less than 1mTorr. The purified concentrate is free from all indigenous heavy molecules which is critical in the following separation and purification processes.

To the purified concentrate, saponification process was carried out in the presence of hydroxide and alcohol. They hydroxides used are sodium hydroxide and potassium hydroxide whereas alcohols used including methanol, ethanol and iso-propanol. The unsaponifiable materials were recovered using hydrocarbon solvents extraction of the reaction mixture such as heptane, hexane, iso-octane and petroleum ether. The hydrocarbon layer was neutralized with copious of water washing and the unsaponifiable matters recovered containing only vitamin E, phytosterols and squalene.

Phytosterols were crystallized out from the unsaponifiable mixture using water/alcohol/hydrocarbon system by heating and cooling processes preferably from 70°C – 85°C to 25°C – 35°C. The crystallized phytosterols were filtered and to the remaining part of the mixture, hydrocarbon solvent and alkyl alcohol was introduced to partitioning the less polar squalene into hydrocarbon layer and the relatively more polar vitamin E into the alkyl alcohol layer. The alkyl alcohols used including methanol and ethanol and hydrocarbon solvents used including hexane, heptane and iso-octane.

DESCRIPTION OF THE DRAWING

Figure 1 shows a schematic representation of the extraction process of the phytonutrients concentrate.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described with reference to Figure 1 and to the following steps as example of the steps involved in the extraction process. The quantities used and parameters used are by way of example only and are not limited thereto unless otherwise stated.

Example 1

5kg of crude palm oil was esterified with 2.5 kg of methanol and 50g of NaOH as catalyst. The methyl esters were separated from glycerol and neutralized by water washing. Methyl esters were subjected to the first short path distillation at temperature of 90°C and pressure of 20mTorr. The residue was then subjected to second short path distillation under operating temperature of 150°C and pressure of 1mTorr to remove all coloured materials/pigments. The light yellowish distillate was subsequently subjected to third short path distillation with temperature of 90°C and pressure of 1mTorr for the production of vitamin E, phytosterols and squalene (phytonutrients) concentrates. The detailed analysis results of the phytonutrients concentrates are shown in Table 1.

Example 2

3 grams of the purified phytonutrients concentrates as obtained from Example 1 or from other sources was saponified using 5ml of 10% KOH and 20ml of ethanol. The mixture was refluxed under nitrogen blanketing for 30 minutes. The reacted mixture was transferred into a separating funnel with 10ml of ethanol, 20ml of hot distilled water and 30ml of hexane. The mixture was shaken and cooled to room temperature leaving hexane layer at the top and aqueous layer at the bottom. The unsaponifiable materials, which is hexane soluble was collected from the top whereas the aqueous layer was further extracted 5 times with 30ml hexane/water of ratio 9:1. The hexane layer recovered was neutralized with water washing and all the solvents was removed by rotary-evaporator and vacuum pump drying. Recovery of vitamin E, phytosterols and squalene are 83%, 93% and 86%. The detailed analysis results are shown in Table 2.

Example 3

0.42 grams of the unsaponifiable materials from saponification of purified phytonutrients concentrates as obtained from Example 2 or from other sources was added with 5ml of ethanol, 5ml of hexane and 0.5 ml of distilled water. The mixture was shaken to a homogeneous stage and settled into two layers. The hexane layer at the top was separated from the ethanol/water layer at the bottom. Solvents were removed using rotary-evaporator and vacuum pump dryer. The concentration of squalene in hexane layer is 41% with recovery of 97% and the concentration of sterols in ethanol layer is 64.7% with recovery of 52.9%. The concentration of vitamin E in hexane and ethanol layers is 12% and 20.4%. The detailed analysis results are shown in Table 3.

Example 4

0.8 grams of the unsaponifiable matters with phytosterols concentration of 39.4% from saponification of purified phytonutrients concentrates as obtained from Example 3 or from other sources was added with 2.5 ml hexane, 0.1 ml methanol and 0.1 ml distilled water. The mixture was heated to 70°C and cooled slowly to 28°C. The solid crystal formed was filtered with suction and washed with copious amount of hexane. The solvents in the filtrate were rotary evaporated and vacuum pump dried. The concentration of phytosterols is 99% with recovery of 63.5%. The detailed analysis results are shown in Table 4.

Example 5

0.73 grams of the unsaponifiable matters with phytosterols concentration of 39.4% from saponification of purified phytonutrients concentrates was added as obtained from Example 4 or from other sources with 3.5 ml hexane, 0.1 ml methanol and 0.1 ml distilled water. The mixture was heated to 70°C and cooled slowly to 28°C. The solid crystal formed was filtered with suction and washed with copious amount of hexane. The solvents in the filtrate were rotary evaporated and vacuum pump dried. The concentration of phytosterols is 99% with recovery of 41.7%. The detailed analysis results are shown in Table 5.

Example 6

0.69 grams of the unsaponifiable matters with phytosterols concentration of 39.4% from saponification of purified phytonutrients concentrates as obtained from Example 5 or from other sources was added with 2.5 ml hexane, 0.05 ml methanol and 0.1 ml distilled water. The mixture was heated to 70°C and slowly cooled to 28°C. The solid crystal formed was filtered with suction and washed with copious amount of hexane. The solvents in the filtrate were rotary evaporated and vacuum pump dried. The concentration of phytosterols is 99% with recovery of 36.8%. The detailed analysis results are shown in Table 6.

Example 7

0.71 grams of the unsaponifiable matters with phytosterols concentration of 54.4% from saponification of purified phytonutrients concentrates as obtained from Example 6 or from other sources was added with 2.5 ml hexane, 0.1 ml methanol and 0.1 ml distilled water. The mixture was heated to 70°C and slowly cooled to 28°C. The solid crystal formed was filtered with suction and washed with copious amount of hexane. The solvents in the filtrate were rotary evaporated and vacuum pump dried. The concentration of phytosterols is 99% with recovery of 41%. The detailed analysis results are shown in Table 7.

Example 8

0.29 grams of the filtrate obtained from Example 5 or other solvents after crystallisation of phytosterols was added with 5ml hexane and 2ml methanol. The mixture was shaken to a homogeneous stage and settled into two layers. The hexane layer at the top was separated from the methanol layer at the bottom. Solvents were removed using rotary-evaporator and vacuum pump dryer. The concentration of vitamin E in methanol layer is 31.3% with recovery of 52.6% and the concentration of squalene in hexane layer is 51% with recovery of 87.5%. The detailed analysis results are shown in Table 8.

Example 9

0.34 grams of the filtrate obtained from Example 7 or other solvents after crystallisation of phytosterols was added with 5ml hexane and 3ml methanol. The mixture was shaken to a homogeneous stage and settled into two layers. The hexane layer at the top was separated from the methanol layer at the bottom. Solvents were removed using rotary-evaporator and vacuum pump dryer. The concentration of vitamin E is 51.2% with recovery of 57.5% and the concentration of squalene in hexane layer is 44.2% with recovery of 95.4%. The detailed analysis results are shown in Table 9.

Example 10

The filtrate of the unsaponifiable matters after crystallisation of phytosterols from purified phytonutrients concentrate was treated with serial partitioning of organic solvents to enhance the concentration of vitamin E and squalene. 0.6g of filtrate was added with 5rnl of hexane and 3ml of methanol. The mixture was chilled to 15°C for 15 minutes. The hexane layer was separated from methanol layer and analysed, lml of hexane was subsequently added to methanol layer and lml of methanol was added to hexane layer. After chilling to 15°C for another 15 minutes, all the hexane and methanol layers were separated. All samples were analysed for vitamin E and squalene contents. The concentration of vitamin E in methanol phase after second partitioning of methanol layer is 79.3% with recovery of 34.9%. The concentration of squalene in hexane phase after second partitioning of hexane layer is 77.2% with recovery of 65.5%. The detailed analysis results are shown in Table 10. The process is described in Figure 2.

Table 1

Sample Code	Percentage (%)									
	FFA	**Esters**	**MG**	**DG**	**TG**	**Squalene**	**Sterols**	**Vitamin E**	**Carotenes**	**Undetermined Products**
Crude Palm Oil Methyl Esters	0.00	98.13	1.11	0.22	0.06	0.07	0.06	0.05	0.06	ND
Residue (1st Stage SPD)	0.00	84.09	9.99	2.87	0.78	0.64	0.67	0.47	0.64	ND
Residue (2nd Stage SPD)	0.00	2.40	0.00	43.57	7.82	0.00	1.11	0.71	4.99	39.40
Residue (3rd Stage SPD)	0.00	5.78	53.69	3.94	0.53	6.56	8.40	3.98	0.12	ND
Distillate (1st Stage SPD)	0.00	99.00	0.05	0.00	0.00	0.00	0.00	0.01	0.00	ND
Distillate (2nd Stage SPD)	0.00	89.40	8.96	0.07	0.00	0.59	0.62	0.41	0.02	ND
Distillate (3rd Stage SPD)	0.00	98.33	0.25	0.00	0.00	0.00	0.00	0.01	0.00	ND

Table 2

Sample Code	Percentage (%)									
	FFA	**Esters**	**MG**	**DG**	**TG**	**Squalene**	**Sterols**	**Vitamin E**	**Carotenes**	**Weight (g)**
Purified Phytonutrients Conc	0	4.48	66.33	0	1.04	6.39	15.06	6.66	0.05	3.03
Unsaponifiable Materials	3.8	0.0	0.0	0.0	2.5	20.5	52.4	20.7	0.2	0.81

Table 3

Sample Code	Percentage (%)									
	FFA	**Esters**	**MG**	**DG**	**TG**	**Squalene**	**Sterols**	**Vitamin E**	**Carotenes**	**Weight (g)**
Unsaponifiable Materials (Starting materials)	3.6	0.0	0.0	0.0	2.9	25.5	49.5	18.2	0.1	0.42
Hexane Layer	2.3	0.0	0.0	0.0	4.9	41.0	39.7	12.0	0.1	0.26
EtOH Layer	5.3	0.0	0.0	0.0	0.3	9.3	64.7	20.4	0.1	0.17

Table 4

Sample Code	Percentage (%)									
	FFA	**Esters**	**MG**	**DG**	**TG**	**Squalene**	**Sterols**	**Vitamin E**	**Carotenes**	**Weight (g)**
Unsaponifiable Materials (Starting materials)	8.8	0.0	0.0	0.3	3.2	33.7	39.4	14.1	0.3	0.79
Filtrate	11.3	0.0	0.0	0.5	3.9	46.9	18.4	17.2	0.5	0.61
Solid	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.19

Table 5

Sample Code	Percentage (%)									
	FFA	Esters	MG	DG	TG	Squalene	Sterols	Vitamin E	Carotenes	Weight (g)
Unsaponifiable Materials (Starting materials)	8.8	0.0	0.0	0.3	3.2	33.7	39.4	14.1	0.3	0.73
Filtrate	10.7	0.0	0.0	0.6	4.2	43.2	24.6	16.2	0.4	0.61
Solid	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.12

Table 6

Sample Code	Percentage (%)									
	FFA	**Esters**	**MG**	**DG**	**TG**	**Squalene**	**Sterols**	**Vitamin E**	**Carotenes**	**Weight (g)**
Unsaponifiable Materials (Starting materials)	8.8	0.0	0.0	0.3	3.2	33.7	39.4	14.1	0.3	0.69
Filtrate	10.0	0.0	0.0	0.4	3.5	41.0	28.9	15.7	0.3	0.59
Solid	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.10

Table 7

Sample Code	Percentage (%)									
	FFA	**Esters**	**MG**	**DG**	**TG**	**Squalene**	**Sterols**	**Vitamin E**	**Carotenes**	**Weight (g)**
Unsaponifiable Materials (Starting materials)	1.8	0.0	0.0	0.0	2.5	18.5	54.4	22.7	0.2	0.71
Crystallized Phytosterols	0.0	0.0	0.0	0.0	0.0	0.0	99.0	0.0	0.0	0.16
Filtrate	3.0	0.0	0.0	0.0	4.8	30.0	35.9	26.2	0.2	0.55

Table 8

Sample Code	Percentage (%)									
	FFA	Esters	MG	DG	TG	Squalene	Sterols	Vitamin E	Carotenes	Weight (g)
Filtrate (Starting materials)	11.7	0.0	0.0	0.0	3.7	42.2	21.4	16.4	0.5	0.29
Hexane Layer	11.3	0.0	0.0	0.4	4.6	51.0	17.4	10.3	0.5	0.21
MeOH Layer	10.2	0.0	0.0	0.0	1.7	23.6	33.0	31.3	0.3	0.08

Table 9

Sample Code	Percentage (%)									
	FFA	**Esters**	**MG**	**DG**	**TG**	**Squalene**	**Sterols**	**Vitamin E**	**Carotenes**	**Weight (g)**
Filtrate (Starting materials)	3.0	0.0	0.0	0.0	4.8	30.0	35.9	26.2	0.2	0.34
Hexane Layer	1.2	0.0	0.0	0.0	9.3	44.2	27.1	17.1	0.2	0.22
MeOH Layer	4.3	0.0	0.0	0.0	0.8	5.4	38.1	51.2	0.1	0.10

Table 10

Sample Code	Percentage (%)									
	FFA	**Esters**	**MG**	**DG**	**TG**	**Sterols**	**Squalene**	**Vitamin E**	**Carotenes**	**Weight (g)**
Filtrate	8.56	0.00	0.00	0.00	0.00	18.20	36.55	36.70	0.30	0.60
Hexane 1	3.12	0.00	0.00	0.00	0.00	8.78	69.10	19.00	0.20	0.25
Hexane 2	4.00	0.00	0.00	0.00	0.00	11.72	54.12	30.16	0.30	0.12
Hexane 3	1.57	0.00	0.00	0.00	0.00	8.70	77.21	12.52	0.30	0.19
Methanol 1	9.92	0.00	0.00	0.00	0.00	15.10	18.18	56.80	0.20	0.31
Methanol 2	1.69	0.00	0.00	0.00	0.00	13.85	5.12	79.33	0.20	0.10
Methanol 3	0.01	0.00	0.00	0.00	0.00	22.10	18.37	59.52	0.10	0.04

CLAIMS

1. A method of extraction of phytosterols, squalene and vitamin E from crude palm oil comprising the steps of: -
   a) conversion of crude palm oil into palm oil methyl esters;
   b) three short path distillation of crude palm oil methyl esters obtained in 1 (a) to yield phytonutrients;
   c) saponification of phytonutrients concentrate from 1(b);
   d) crystallisation of phytosterols;
   e) solvents partitioning of vitamin E and squalene.

2. A method of extracting phytosterols squalene or Vitamin E as claimed in Claim 1, wherein a first short path distillation is carried out at temperature of 70°C to 120°C and pressure between 10mTorr to 50mTorr.

3. A method of extracting phytosterols squalene and Vitamin E as claimed in Claim 1, wherein a second short path distillation is carried out on the distillate obtained in Claim 2 at temperature of 130°C to 200°C and pressure less than 1mTorr.

4. A method of extracting phytosterols squalene and Vitamin E as claimed in Claim 1 wherein a third short path distillation is carried out on the distillate obtained in Claim 3 at temperature below 120°C and pressure less than 1mTorr.

5. A method as claimed in Claim 1, wherein the saponification process of phytonutrients concentrate is carried out using potassium hydroxide or sodium hydroxide at 10% concentration and refluxed in alcohol for 30 minutes to one hour under inert gas blanketing.

6. A method as claimed in Claim 5, wherein the inert gas is nitrogen.

7. A method as claimed in Claim 5, wherein unsaponifiable matters is mixed with hydrocarbon solvent, short chain alcohol and water of different ratios.

8. A method as claimed in Claim 5, wherein the unsaponifiable matters is mixed with hydrocarbon solvent, short chain alcohol and water of ratio 25:1:1 and heated to temperature of 65°C to 85°C and slowly cooled to temperature of 10°C to 30°C to crystallize phytosterols.

9. A method as claimed in Claim 8 wherein the filtrate is mixed with hydrocarbon solvent and short chain alcohol of ratio 5:3 to partition the non-polar squalene into hydrocarbon layer and polar vitamin E into alcohol layer.

10. A method as claimed in Claims 5, 7, 8 and 9, wherein the hydrocarbon solvents including heptane, hexane and iso-octane and short chain alcohols including methanol, ethanol, butanol and iso-propanol.

11. Vitamin E, squalene or phytosterols as extracted as in any of the Claims 1 to 10.

12. A method of extraction of phytosterols, squalene and vitamin E from crude palm oil comprising the steps of: -

i. conversion of crude palm oil into palm oil methyl esters;

ii. first stage short path distillation carried out on the methyl esters obtained in step (i) above at a temperature of 70°C to 120°C and pressure between 10mTorr to 50mTorr;

iii. second stage short path distillation carried out on the distillate obtained in step (ii) above at a temperature of 130°C to 200°C and pressure less than 1mTorr;

iv. third stage short path distillation carried out on the distillate obtained in step (iii) above at a temperature below 120°C and pressure less than 1mTorr;

v. saponification of the distillate obtained in step (iv) above carried out using potassium hydroxide or sodium hydroxide at 10% concentration and refluxed in alcohol for 30 minutes to one hour under nitrogen blanketing;

vi. mixing the unsaponifiable matters in step (v) above with hydrocarbon solvent, short chain alcohol and water of ratio 25:1:1 and heating mixture to temperature of 65°C to 85°C and cooling slowly to temperature of 25°C to 30°C to crystallize phytosterols;

vii. mixing filtrate obtained in step (vi) above with a hydrocarbon selected from the group consisting of heptane, hexane or iso-octane and a short chain alcohol selected from the group consisting of methanol, ethanol, butanol or iso-propanol in ratio 5:3 to partition non-polar squalene into hydrocarbon layer and polar vitamin E into alcohol layer;

viii. separating two layers and subsequently adding hydrocarbon selected in step (viii) into short chain alcohol layer selected in step (viii) and short chain alcohol selected in step (viii) into hydrocarbon layer to further partition the vitamin E and squalene.

ix. extracting squalene from the hydrocarbon layer and extracting vitamin E from the alcohol layer.

13. Phytosterol crystals as obtained in Claim 8.

ABSTRACT

EXTRACTION OF PALM VITAMIN E, PHYTOSTEROLS AND SQUALENE FROM PALM OIL

Phytosterols, squalene and Vitamin E are recovered from phytonutrients concentrate derived from crude palm oil by the disclosed invention via esterification, transesterification, vacuum distillation, saponification, crystallization and organic solvents partitioning. Crude palm oil is subjected to esterification and transesterification for the production of crude palm oil methyl esters. Phytonutrients concentrate containing phytosterols, squalene, Vitamin E and unreacted monoglycerides is recovered from crude palm oil methyl esters by multi-stages vacuum distillation in which components with higher molecular weight are filtered during second stage vacuum distillation. The purified phytonutrients concentrate is subjected to saponification process and the unsaponifiable matter is added to a combination of solvents for crystallization of phytosterols. The filtrate enriched in squalene and Vitamin E is separated to its individual squalene-rich layer and vitamin E-rich layer via organic solvents partitioning.

Figure 1

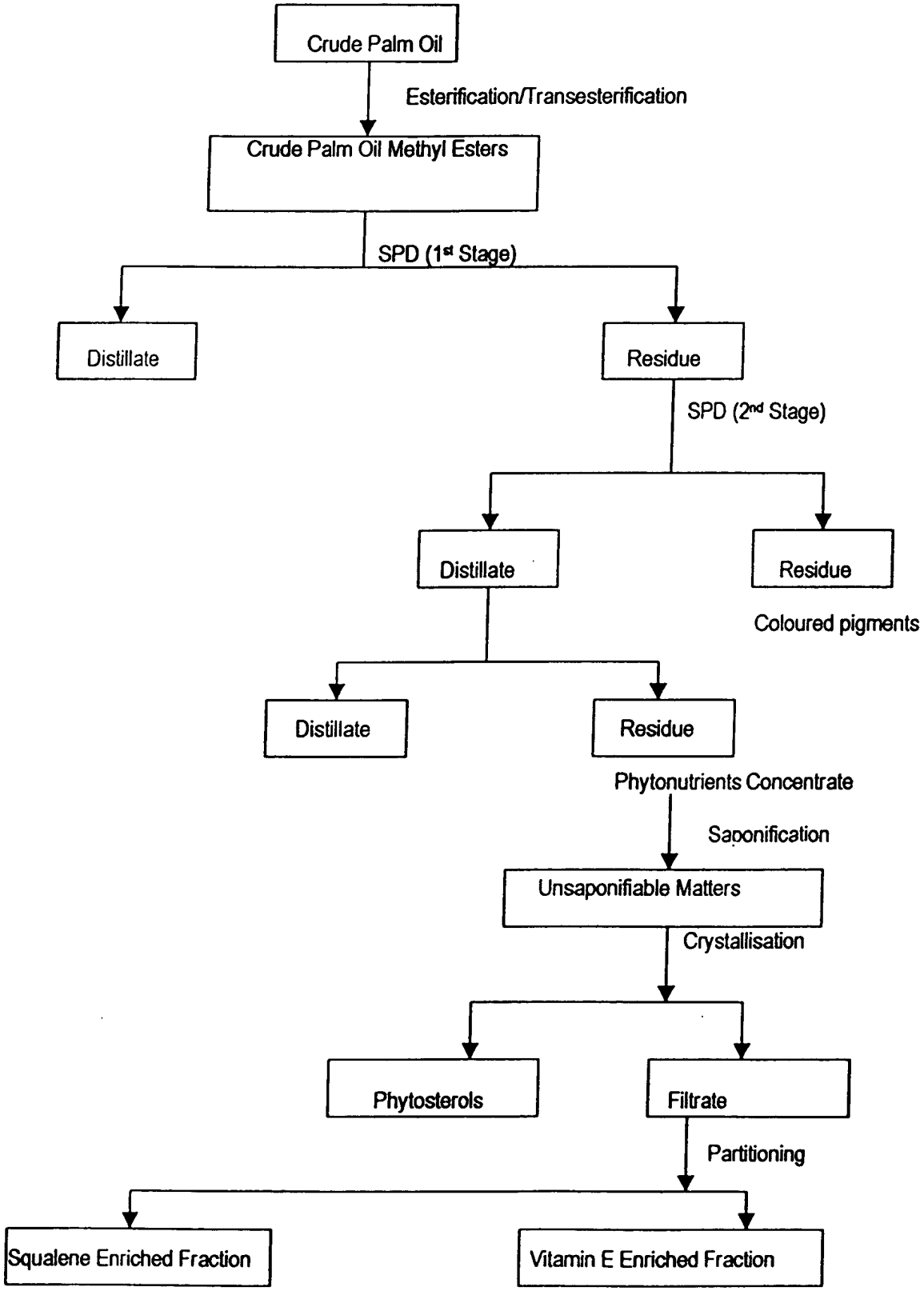

Crude Palm Oil
Esterification/Transesterification
Crude Palm Oil Methyl Esters
SPD (1st Stage)
Distillate
Residue
SPD (2nd Stage)
Distillate
Residue
Coloured pigments
Distillate
Residue
Phytonutrients Concentrate
Saponification
Unsaponifiable Matters
Crystallisation
Phytosterols
Filtrate
Partitioning
Squalene Enriched Fraction
Vitamin E Enriched Fraction

FIGURE 1

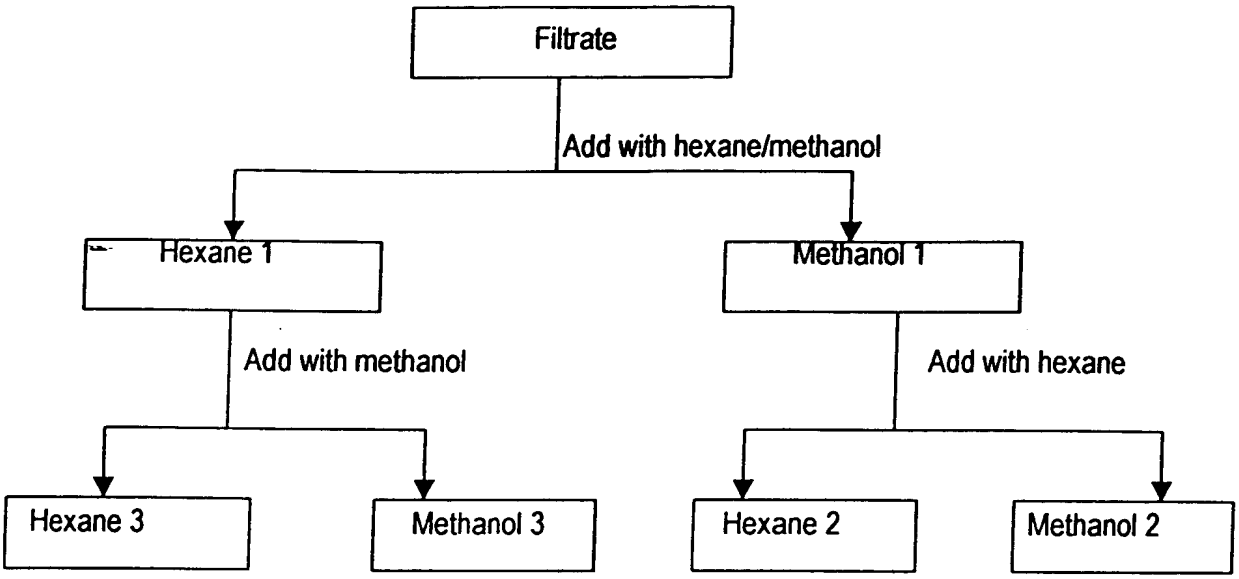

Filtrate
Add with hexane/methanol
Hexane 1
Methanol 1
Add with methanol
Add with hexane
Hexane 3
Methanol 3
Hexane 2
Methanol 2

Figure 2